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(54) Title: USE OF NITRIC OXIDE DONORS IN MEDICINE

(57) Abstract

The use of an NO donor for the treatment and/or prophylaxis of restenosis and/or thrombotic conditions involving platelets, is disclosed. Preferred NO donors are S-nitroso compounds of the formula: R-SNO, wherein R is one or more amino acid derived fragments.

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USE OF NITRIC OXIDE DONORS IN MEDICINE

The present invention relates to the use of nitric oxide (NO) donors, in combating restenosis and/or thrombotic conditions involving platelets.

Restenosis can occur following a number of invasive surgical techniques, for example, transplant surgery, vein grafting, coronary by-pass grafting, arteriovenous anastamosis and most commonly, following angioplasty.

The mechanism underlying the development of restenosis is still unclear. The key process appears to be local damage on a vessel wall caused during one of the above mentioned invasive surgical techniques. It has been suggested that the injury inflicted during angioplasty, for example by the use of balloon catheters, wherein stenoses are opened up by compressing and/or tearing the plaque on the vessel walls, induces a reparative and proliferative response which in some cases, becomes exuberant enough to encroach on the residual lumen of the angioplasty site and produces clinical restenosis (Journal of the American College of Cardiology (1987) 9 (4), p.834-848). Alternatives to the balloon catheter, such as pulsed lasers and rotary cutters, have been developed with a view to reducing or preventing restenosis following angioplasty, but have met with limited success.

Restenosis tends to develop over a period ranging from 1 to 6 months after angioplasty has been performed. It usually presents itself as the recurrence of angina-like symptoms, a decrease in the threshold for effort angina, or acute events (sudden death, myocardial infarction, and unstable angina along with the need for bypass

surgery). It is thought to occur in greater than 30% of cases and in many patients may be asymptomatic.

A large number of therapeutic approaches have been used in an attempt to modify the restenosis process. These include the use of antiplatelet agents, anticoagulants, vasodilators, angiotensin-converting enzyme inhibitors, fish oil and cholesterol-lowering agents, and antiproliferative agents. None of the treatments or approaches tried to date have been successful in preventing restenosis in humans.

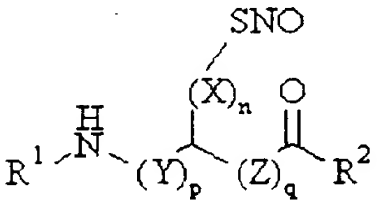
It has now been found that nitric oxide (NO) donors have a particular utility as anti-thrombotic agents and in the treatment and/or prophylaxis of restenosis in mammals. Accordingly the present invention provides the use of an NO donor in the manufacture of a medicament for combating restenosis and/or thrombotic conditions involving platelets. There is further provided a method of treatment and/or prophylaxis of restenosis and/or thrombotic conditions involving platelets comprising administering to a mammal in need thereof an effective amount of a NO donor.

NO donors may be used whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to treat or prevent the formation of thrombi in mammals, including man. For example, they may be used in the treatment and prevention of myocardial infarcts, in the treatment of peripheral vascular disease, to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, as additives to blood, blood products, blood substitutes, and other fluids which are used in extra-corporeal circulation and the fusion of isolated body portions, to treat complications of arteriosclerosis and conditions such as atherosclerosis,

blood clotting defects due to lipemia, as well as other clinical conditions in which the underlying etiology is associated with lipid imbalance of hyperlipidemia, and in the treatment of disseminated intravascular coagulation. In particular however, the NO donors are of use in the prevention of restenosis following transplant surgery, vein grafting, coronary by-pass grafting and in particular, following angioplasty.

By the term "NO donor" is meant any compound which is capable of liberating NO *in vivo*. Whilst any compound which is a NO donor can be used according to the present invention, a preferred group of compounds are S-nitroso compounds of the formula R-SNO wherein R is one or more amino acid derived fragments.

In one aspect, the NO donors of the present invention are a group of compounds of formula (I)



(I)

wherein

n is 0 or 1; X is a C_{1-6} straight or branched alkylene chain;

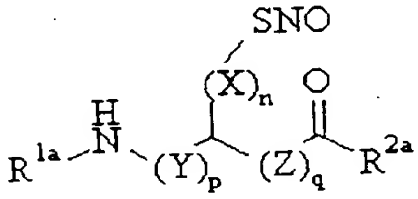
p and q are independently 0 or 1; Y and Z may be the same or different and are each a C_{1-4} hydrocarbyl chain optionally substituted by one or more groups R^4 and R^5 wherein R^4 and R^5 may be the same or different and are selected from hydrogen, C_{1-4} alkyl or C_{6-10} aryl;

R^1 is hydrogen or a group COR^3, wherein R^3CO_2H is a natural L-amino acid (other than cysteine) and/or the D-isomer thereof;

R^2 is OH or a group NR^6R^7, wherein HNR^6R^7 is a natural L-amino acid (other than cysteine) and/or the D-isomer thereof;

and salts, esters and amides thereof.

In a further aspect, the NO donors of the present invention are a group of compounds of formula (IA)



(IA)

wherein n, p, q, X, Y and Z are as hereinbefore defined;

R^{1a} is hydrogen or a group COR^{3a} wherein R^{3a} is a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2;

R^{2a} is OH or a group $NR^{6a}R^{7a}$ wherein R^{6a} is a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2 or a C_{1-4} alkyl group optionally substituted by COOH; and R^{7a} is hydrogen or a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2 or a C_{1-4} alkyl group optionally substituted by COOH; or R^{6a} and R^{7a} may be joined to form a 5- or 6- membered heterocyclic ring;

and salts, esters and amides thereof.

A particularly preferred NO donor for use according to the present invention is S-nitroso-glutathione (GSNO) and all salts, esters and amides thereof.

The NO donors of the present invention may be administered before, coincidentally with or at any time after invasive surgery, such as angioplasty. It is preferred that the administration of the NO donor is commenced before or coincidentally with surgery, and most preferably before surgery.

Whilst it may be possible for the NO donors to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further feature of the present invention we provide pharmaceutical formulations for use in the methods of the invention comprising at least one NO donor, or a pharmaceutically acceptable salt, ester or amide thereof, together with one or more pharmaceutically acceptable carriers or excipients and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The effective amount of active ingredient required is from 10 mg/day to 500mg/day, suitably 20mg/day to 360mg/day, depending on the particular NO donor administered. Suitably, sufficient compound is given which will liberate 50μmol to 1mmol of NO/day. Pharmaceutical formulations may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose.

Pharmaceutical formulations may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intraarterial, intracoronary, intraarticular or intradermal) route. Such formulations may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the carrier(s) or excipient(s). Most suitably the NO donors are administered orally (e.g. sub-lingually), topically (e.g. by means of a patch) or parenterally (e.g. by infusion).

Pharmaceutical formulations adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

Pharmaceutical formulations adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3(6), 318 (1986).

Pharmaceutical formulations adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

Pharmaceutical formulations adapted for nasal administration wherein the carrier is a solid include a

coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostatis and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The present invention will now be described by way of example only:

Methods

13 patients were studied undergoing left coronary percutaneous transluminal coronary angioplasty (PTCA) for stable angina. They were divided into 2 groups, with 7 controls and 6 patients who received a continuous intracoronary infusion of 40nmol/minute GSNO via the guide catheter. This started 10 minutes before PTCA and continued for 10 minutes following the procedure. All patients received standard premedication of aspirin 300mg p.o. and buccal glyceryl trinitrate (GTN) 3mg. Sodium heparin 20,000 units was given via the femoral artery at the start of the procedure. An 8F catheter was inserted into the coronary sinus and blood taken into a syringe containing anticoagulant (1:9 v:v. 3.15% trisodium citrate) before PTCA and for 10 minutes following PTCA. Between samples the coronary sinus catheter was continuously flushed with heparinised 0.9% saline.

The procedure was standardised, such that baseline samples were collected before positioning of the angioplasty guide wire and balloon. In the GSNO-treated group, a further pre-PTCA sample was taken 10 minutes after starting the GSNO infusion. GSNO infusion was then continued throughout the PTCA procedure and for 10 minutes afterwards. Timing following PTCA started at the end of the final inflation, at which point the balloon was withdrawn. No contrast medium was injected after the first inflation until the final collection at 10 minutes. Blood pressure was monitored via the guide catheter.

The blood samples were incubated with fluorescein isothiocyanate (FITC)-labelled anti-human P-selectin (Immunotech) or GPIIIa (Dako) monoclonal antibodies (1), with an isotype-matched non-specific FITC-conjugated mouse IgG (Dako) as a negative control. Samples were analysed in duplicate using a FACScan (Becton Dickinson) flow cytometer calibrated daily with fluorescent microbead standards (Becton Dickinson). The platelet population was identified on the basis of size and granularity of cells, and specific binding of P-selectin and GPIIIa (part of the GPIIb/IIIa complex) were measured (figure 1). P-selectin is only expressed following platelet activation with alpha granule secretion. Antibody binding was therefore measure, after subtracting non-specific fluorescence, as the percentage of platelets positive for the P-selectin antibody. In contrast, all platelets express GPIIb/IIIa. In addition, GPIIb/IIIa is present on the surface-connected canalicular system and the number of receptors expressed increases with platelet activation. Therefore GPIIb/IIIa antibody binding was measured as the relative change in fluorescence intensity per platelet (figure 2).

Results are expressed as mean ± SEM. Statistical differences were determined using the Wilcoxon test for paired data and the Mann-Whitney U test for unpaired data and $p<0.05$ was taken as statistically significant.

Results

The two groups of patients were closely matched. All angioplasties were performed on lesions with at least 70% stenosis. Angiography at the end of the study confirmed successful PTCA in all cases, defined as less than 50% residual stenosis with at least a 20% reduction in the original stenosis.

In the control group, platelet expression of P-selectin and GPIIb/IIIa increased following PTCA, with a peak at 5 minutes after the procedure (baseline: P-selectin was expressed by 1.6±0.2% of platelets, GPIIb/IIIa relative fluorescence 3.2±0.4 arbitrary units; 5 minutes post-PCTA: P-selectin 4.7±1.1%, GPIIb/IIIa 4.0±0.3, p=0.036 for each compared with baseline). A 10 minute intracoronary infusion of GSNO immediately prior to PTCA caused no change in blood pressure (baseline:135±15/67±6 mmHg; after 10 minutes infusion of GSNO: 132±16/65±7 mmHg. In the GSNO-treated group, P-selectin expression fell during GSNO infusion, with no increase following PTCA (baseline: 1.8±0.6%; pre-PTCA after 10 minute infusion of GSNO: 1.1±0.4%, p<0.05 compared with baseline; 5 minutes post PTCA: 0.7±0.1%, p<0.05 compared with baseline, p=0.0034 compared with change in control group) (figure 1). GPIIb/IIIa expression, although not significantly reduced during GSNO infusion, did not increase following PTCA (baseline 3.4±0.8; 5 minutes post PTCA: 3.3±0.9, p=NS compared with baseline, p=0.026 compared with change in control group).

Discussion

A time-dependent increase in platelet P-selectin and GPIIb/IIIa expression following PTCA has been demonstrated. This was maximal 5 minutes after PTCA, with an increase in P-selectin still detectable at 10 minutes. A previous study suggested that an increase in P-selectin expression was found only in association with angiographically-suspected dissection of the coronary artery (2). The present study shows a rise in P-selectin in all cases, regardless or angiographic evidence of coronary dissection. Our findings show that PTCA is always accompanied by platelet activation, which is not prevented even in the presence of standard anti-thrombotic therapy with aspirin, glyceryl trinitrate

(GTN) and heparin.

We have supplemented endogenous NO by infusing GSNO during PTCA and have shown that platelet activation, occurring in the presence of conventional anti-platelet treatment, was prevented by infusion of this NO donor. Moreover, inhibition of platelet activation occurred at a dose of GSNO that had no effect on blood pressure. Clinically-used nitrates, which release relatively little NO to platelets (3), have a predominantly vasodilator effect and cause hypotension at doses required for platelet inhibition (4-6). S-nitrosoglutathione, by comparison, has a more potent anti-platelet action with a relatively weak vasodilator effect.

The results, showing that GSNO is a potent and effective inhibitor of PTCA-induced platelet activation, have important clinical implications since currently-used anti-platelet treatments are not satisfactory. Indeed although anti-platelet treatment with aspirin during PTCA reduces thrombus formation it does not completely prevent it (7). Aspirin is also ineffective in preventing restenosis (8). Similarly prostacyclin, which has a potent anti-aggregatory effect but only inhibits platelet adhesion at much higher doses (9), does not inhibit platelet activation or reduce the rate of restenosis following PTCA (10). Thus GSNO and/or GSNO-like compounds, which enable the effects of NO to be targeted, may represent an important new line of therapy for prevention of acute vessel closure and restenosis after PTCA.

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CLAIMS

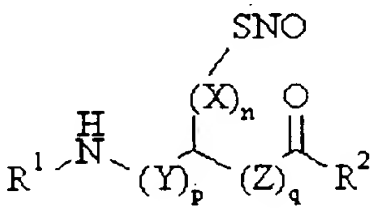
1. The use of a nitric oxide (NO) donor in the manufacture of a medicament for combating restenosis and/or thrombotic conditions involving platelets.

2. The use of a NO donor in the manufacture of a medicament for the treatment and/or prevention of restenosis.

3. The use of an NO donor in the manufacture of a medicament for the treatment and/or prophylaxis of thrombotic conditions involving platelets.

4. The use according to any of the preceding claims wherein the NO donor is a S-nitroso compound of the formula R-SNO wherein R is one or more amino acid derived fragments.

5. The use according to any one of claims 1 to 3 wherein the NO donor is a compound of the formula (I)



(I)

wherein

n is 0 or 1; X is a C_{1-6} straight or branched alkylene chain;

p and q are independently 0 or 1; Y and Z may be the same or different and are each a C_{1-4} hydrocarbyl chain optionally substituted by one or more groups R^4 and R^5 wherein R^4 and R^5 may be the same or different and are

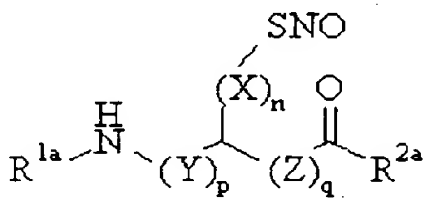
selected from hydrogen, C_{1-4} alkyl or C_{6-10} aryl;

R^1 is hydrogen or a group COR^3, wherein R^3CONH_2 is a natural L-amino acid (other than cysteine) and/or the D-isomer thereof;

R^2 is OH or a group NR^6R^7, wherein HNR^6R^7 is a natural L-amino acid (other than cysteine) and/or the D-isomer thereof;

or a salt, ester or amide thereof.

6. The use according to any one of claims 1 to 3 wherein the NO donor is a compound of formula (IA)



(IA)

wherein n, p, q, X, Y and Z are as defined in claim 5;

R^{1a} is hydrogen or a group COR^{3a} wherein R^{3a} is a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2;

R^{2a} is OH or a group $NR^{6a}R^{7a}$ wherein R^{6a} is a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2 or a C_{1-4} alkyl group optionally substituted by COOH; and R^{7a} is hydrogen or a C_{1-8} hydrocarbyl group which is optionally substituted by one or two substituents which may be the same or different and are selected from OH, COOH, NH_2 or a C_{1-4} alkyl group optionally substituted by COOH; or R^{6a} and

R^{7a} may be joined to form a 5- or 6- membered heterocyclic ring;

or a salt, ester or amide thereof.

7. The use according to any of the preceding claims wherein the NO donor is S-nitroso-glutathione (GSNO) or all salts, esters and amides thereof.

8. A method of combating restenosis and/or thrombotic conditions involving platelets comprising administering to a mammal in need thereof an effective amount of a NO donor.

9. A method of treatment and/or prevention of restenosis comprising administering to a mammal in need thereof an effective amount of a NO donor.

10. A method of treatment and/or propylaxis of thrombotic conditions involving platelets comprising administering to a mammal in need thereof an effective amount of a NO donor.

11. A method as claimed in any of claims 8 to 10 wherein the NO donor is as defined in any of claims 4 to 7.

12. A composition comprising a NO donor as defined in any of claims 4 to 7 together with a pharmaceutically acceptable carrier or excipient for use in combating restenosis and/or thrombotic conditions involving platelets.

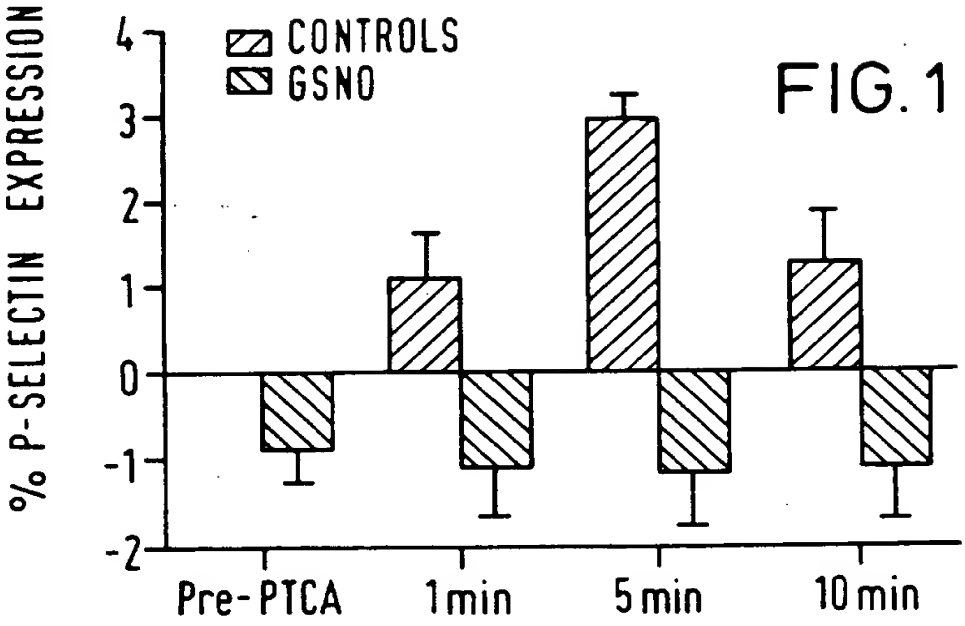

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FIG. 1

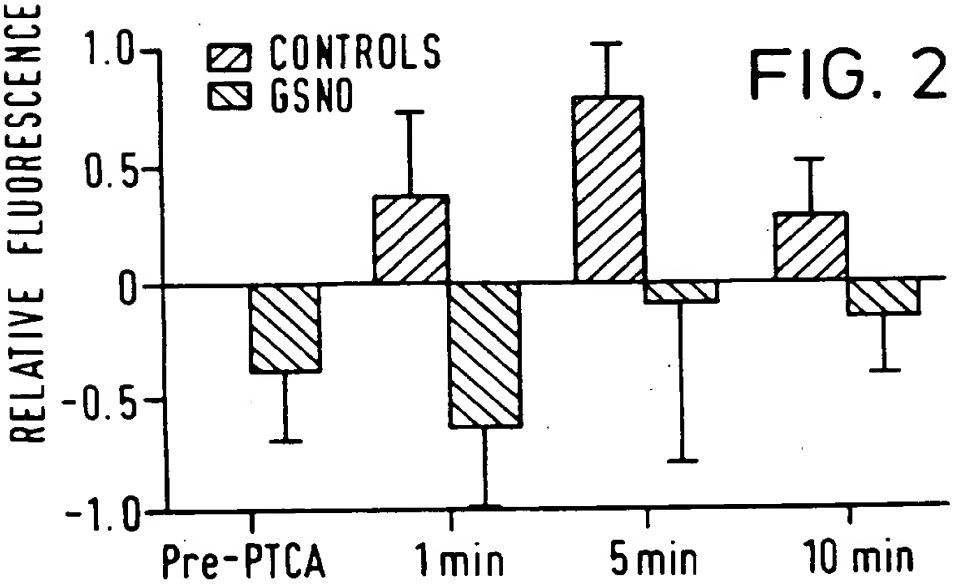
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FIG. 2

INTERNATIONAL SEARCH REPORT

Int[illegible]nal Appli[illegible] No
PCT/GB 95/02745

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/00 A61K31/195

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,O	67TH SCIENTIFIC SESSIONS OF THE AMERICAN HEART ASSOCIATION, DALLAS, TEXAS, USA, NOVEMBER 14-17, 1994. CIRCULATION, vol. 90, no. 4pt2, October 1994 page 1552 E.J.LANGFORD ET AL. 'Activation of Platelets Induced by Coronary Angioplasty is Inhibited by S-nitrosoglutathione' see abstract --- -/--	1-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 1996	19. 04. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Theuns, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO,A,92 18002 (BRIGHAM AND WOMEN'S HOSPITAL) 29 October 1992 see the whole document	1-12

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X,P	CA,A,2 106 105 (L.K.KEEFER ET AL.) 15 March 1995 see the whole document	1-12
X	WO,A,89 12627 (BRIGHAM AND WOMEN'S HOSPITAL) 28 December 1989 see the whole document	1-12
X,P	WO,A,95 24898 (COMEDICUS, INCORPORATED) 21 September 1995 see the whole document	1-12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB95/02745

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 8-11 are directed to a method of treatment of the human/animal body, the search has been based on the alleged effects of the compound/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/02745

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/02745

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